

Evaluation of the Genotypic Prediction of HIV-1 Coreceptor Use versus a Phenotypic Assay and Correlation with the Virological Response to Maraviroc: the ANRS GenoTropism Study[▽]

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Genotypic algorithms for prediction of HIV-1 coreceptor usage need to be evaluated in a clinical setting. We aimed at studying (i) the correlation of genotypic prediction of coreceptor use in comparison with a phenotypic assay and (ii) the relationship between genotypic prediction of coreceptor use at baseline and the virological response (VR) to a therapy including maraviroc (MVC). Antiretroviral-experienced patients were included in the MVC Expanded Access Program if they had an R5 screening result with Trofile (Monogram Biosciences). V3 loop sequences were determined at screening, and coreceptor use was predicted using 13 genotypic algorithms or combinations of algorithms. Genotypic predictions were compared to Trofile; dual or mixed (D/M) variants were considered as X4 variants. Both genotypic and phenotypic results were obtained for 189 patients at screening, with 54 isolates scored as X4 or D/M and 135 scored as R5 with Trofile. The highest sensitivity (59.3%) for detection of X4 was obtained with the Geno2pheno algorithm, with a false-positive rate set up at 10% (Geno2pheno10). In the 112 patients receiving MVC, a plasma viral RNA load of <50 copies/ml was obtained in 68% of cases at month 6. In multivariate analysis, the prediction of the X4 genotype at baseline with the Geno2pheno10 algorithm including baseline viral load and CD4 nadir was independently associated with a worse VR at months 1 and 3. The baseline weighted genotypic sensitivity score was associated with VR at month 6. There were strong arguments in favor of using genotypic coreceptor use assays for determining which patients would respond to CCR5 antagonist.

During the entry process of HIV-1 in the target cell, the interaction of the viral surface glycoprotein gp120 with a cellular chemokine receptor, the coreceptor, is an essential step, besides attachment to the CD4 receptor, and precedes the fusion of the viral envelope to the cell membrane. The V3 hypervariable loop of gp120 is involved in coreceptor binding. Two coreceptors are most commonly used *in vivo*: CCR5 and CXCR4 (1). Viral coreceptor use (i.e., usage of either CCR5 or CXCR4) differs between viral isolates. If CCR5-using iso-

lates (R5 isolates) are by far predominant at the early stage of early infection and seem to be selected during HIV-1 transmission, CXCR4 usage (X4 tropism) will become more prevalent as the infection progresses, with approximately half of X4 or dual or mixed (D/M) tropism in antiretroviral-experienced patients with advanced HIV-1 disease (10).

Maraviroc (MVC), a CCR5 inhibitor, binds specifically to CCR5 and blocks HIV-1 binding to this coreceptor (5). MVC has shown a potent antiviral effect in antiretroviral-experienced patients with R5 HIV-1 infection in a placebo-controlled trial (7) and is currently prescribed in this indication. As MVC has shown little activity in patients with X4 viruses, the determination of HIV-1 coreceptor use has become mandatory before the prescription of CCR5 inhibitors (8).

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Two different methods can be used for determining HIV-1 coreceptor use. Phenotypic assays (4, 11, 17, 18) are based on recombinant virus assays or replication-defective pseudoviruses. They have been widely used but are expensive and time-consuming and require specialized facilities and personnel. Several bioinformatic tools have been proposed to predict coreceptor usage by interpretation of genotypic data—mainly through the use of V3 loop sequencing (9). It is now of importance to evaluate the performance of these genotypic algorithms, for predicting both HIV-1 coreceptor use and virological response (VR) to CCR5 inhibitors. We present here the results of the GenoTropism Study, a multicentric, prospective study aimed at evaluating the genotypic prediction of HIV coreceptor use versus a phenotypic assay and establishing correlations between genotypic tools and the virological response to MVC.

MATERIALS AND METHODS

Study population. The patients included in this study were screened for the Maraviroc Expanded Access Program (MVC EAP) in France between January, 2007 and August 2008 and received MVC associated with an optimized background therapy if the result of the phenotypic assay for coreceptor use determination was CCR5, using a previously validated assay (Trofile; Monogram Biosciences) (18). For the last group of patients included, a modified version with an optimized sensitivity of the assay was performed (16). All screened patients were included in the genotype-phenotype comparison, whereas only patients treated with MVC were included in the study correlating VR to the genotypic results.

Inclusion criteria for the MVC EAP were HIV-1 infection, ≥ 18 years of age, previous antiretroviral therapy, and virological failure with a plasma HIV-1 RNA load of $>1,000$ copies/ml. The patients were included in the GenoTropism Study in 18 participating centers in France. Sociodemographic data, clinical data, and treatment histories were collected for all enrolled patients at the screening date. MVC-treated patients were followed up at baseline (month 0 [M0]) and at months 1, 3, and 6 (M1, M3, and M6, respectively) on MVC-containing regimens. The patients had signed the MVC EAP informed consent form and were specifically informed about their participation in the GenoTropism Study. The study was approved by the Comité Consultatif de Traitement de l'Information dans la Recherche Scientifique et Médicale and the Commission Nationale Informatique et Libertés.

Virological methods. (i) **gp120 sequence analysis and genotypic prediction of coreceptor use.** The gp120 sequence analysis comprising the complete V3 loop sequence was performed from plasma sampled at the date of screening of the MVC EAP, when a sample was also sent for the Trofile assay. PCR primers and conditions and sequencing primers are described in the ANRS (Agence Nationale de Recherches sur le SIDA et les Hépatites Virales, Paris, France) consensus techniques (<http://www.hivfrenchresistance.org>). The bulk V3 sequences were determined by all participant laboratories. Sequences in the FASTA format including possibilities of mixed populations were sent to a central laboratory, where all possible combinations of V3 amino acid sequences present as a result of nucleotide mixtures were considered.

The V3 sequences were then interpreted using 13 different bioinformatics predictors of coreceptor use. The bioinformatic tools C4.5, C4.5 with p8-p12, PART, SVM, and Charge Rule are available at <http://genomiac2.ucsd.edu:8080/wetcat/v3.html>; PSSM_{R5X4} and PSSM_{SINSL} are available at <http://indra.mullins.microbiol.washington.edu/pssm>. Geno2pheno is available at <http://coreceptor.bioinf.mpi-inf.mpg.de/index.php>. For PSSM, the coreceptor use prediction was based on subtype B. For the Geno2pheno website, the false-positive rates (FPRs) for prediction of X4 variants were 5% or 10%. The Geno2pheno interpretation was made with the “clonal” data set (only using bulk sequence analysis) with FPRs of 5% (Geno2pheno5) and 10% (Geno2pheno10) and with the “clinical” data set with an FPR of 10% (Geno2pheno10Clin). In the latter case, the CD4 nadir value, when available, and the viral load at screening were also taken into account.

Finally, three combinations of algorithms were also tested, by scoring X4 when the prediction was X4 with any algorithm: PSSM_{R5X4} with a Geno2pheno FPR of 5% or 10% or all available online algorithms. In all cases, HIV-1 isolates were classified as R5- or X4-tropic, with the latter including pure X4 and mixed R5 and/or X4 isolates on the basis of the analysis of multiple genotypic populations.

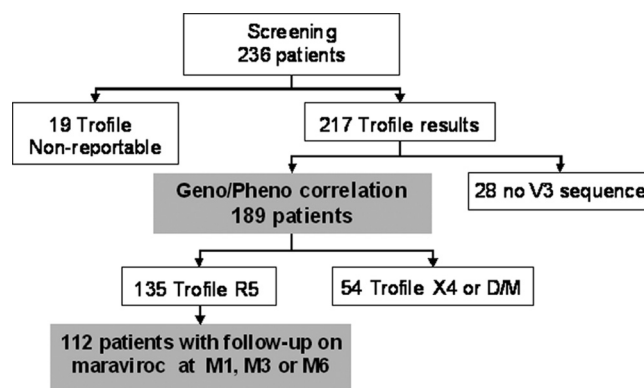


FIG. 1. Study design.

(ii) **Genotypic resistance analysis and GSS.** Sequences of the protease and reverse transcriptase (RT) genes were determined at baseline MVC in each laboratory using the ANRS consensus technique, (<http://www.hivfrenchresistance.org>), the Siemens TruGene kit, and the Abbott ViroSeq kit or an in-house method. The genotypic resistance analysis was interpreted using the ANRS algorithm, as updated in July 2008 (<http://www.hivfrenchresistance.org>). The weighted genotypic sensitivity score (wGSS) was calculated as previously reported (C. Boucher, J. M. Schapiro, D. R. Kuritzkes, J. M. Llibre, M. Lewis, P. Simpson, C. Delogne, V. Sharma, A. Parliyan, D. Chapman, M. Perros, H. Valdez, and M. Westby, presented at the XVIII International HIV Drug Resistance workshop, Fort Myers, FL, 2009). Briefly, one nucleoside RT inhibitor was scored as 0.5 when the virus was sensitive and 0 when the virus was possibly resistant or resistant. A nonnucleoside RT inhibitor, a fusion inhibitor (enfuvirtide), or an integrase inhibitor (raltegravir) was scored as 1 when the virus was sensitive and 0 when the virus was resistant or possibly resistant. A boosted protease inhibitor was scored as 1 when the virus was sensitive, 0.5 when the virus was possibly resistant, and 0 when the virus was resistant. The wGSS was the sum of the scores obtained for the drugs coprescribed with MVC.

(iii) **HIV-1 subtype analysis.** HIV-1 subtype was determined by phylogenetic analysis of RT and gp120 sequences. The nucleotide sequences were aligned by ClustalW1.74 with known reference strains of groups M, N, and O (http://www.hiv.lanl.gov/content/hiv-db/SUBTYPE_REF/align.html). Phylogenetic trees were inferred using the neighbor-joining method and two Kimura parameters with 1,000 bootstrap values.

(iv) **Statistical analyses.** The first part of the study was performed to correlate HIV-1 coreceptor use results between the Trofile assay and different genotypic algorithms, while the second part was performed to determine the factors associated with VR to MVC in patients receiving an MVC-containing regimen on the basis of the Trofile assay. The sensitivity and specificity for predicting CXCR4 were computed for patients included in the first part of our study.

Three virologic responses were defined at three distinct time points. VR was defined as a reduction of at least 1 log₁₀ copy/ml and/or an HIV RNA level of <50 copies/ml at month 1 (M1), a reduction of at least 1.5 log₁₀ copies/ml and/or an HIV-RNA level of <50 copies/ml at M2, and an HIV RNA level of <50 copies/ml at M6. The impact of baseline HIV RNA, baseline CD4 cell count, nadir CD4 cell count, wGSS, and genotypic predictions of CXCR4 on the VR to MVC was investigated. A logistic regression model was performed to search for independent predictive factors associated with VR to MVC. A stepwise selection procedure was used to build final multivariate models.

Nucleotide sequence accession numbers. The reverse transcriptase, protease, and gp120 sequences from the patients in this study were given GenBank accession no. HM035546 to HM035976.

RESULTS

Study population. The study design is described in Fig. 1. During the screening period, 236 patients were screened for the MVC EAP, and corresponding plasma samples were sent for the Trofile assay. The Trofile results were nonreportable for 19 patients and were thus available for 217 patients. Within these 217 patients, 28 patients had no gp120 sequence deter-

TABLE 1. Patients' characteristics at screening and at baseline maraviroc

Characteristic ^a	Result at:	
	Screening (<i>n</i> = 189)	Baseline MVC (<i>n</i> = 112)
General		
Male (%)	78.1	77.5
Median age, yr (IQR)	45.3 (41.8–50.7)	45.7 (42.1–51.2)
Median CD4 cell count/ μl (IQR)	247 (110–396)	257 (123–394)
Median nadir CD4 cell count/μl (IQR)	75 (14–160)	99 (27–177)
Median plasma HIV-1 RNA log ₁₀ copies/ml (IQR)	4.2 (3.5–4.8)	4.2 (3.4–4.9)
Previous antiretroviral treatment		
Median no. of NRTIs (IQR)	6 (5–7)	6 (5–7)
Median no. of NNRTIs (IQR)	1 (1–2)	1 (1–2)
Median no. of PIs (IQR)	4 (3–6)	4 (3–6)
Enfuvirtide (%)	45.0	45.0
Raltegravir (%)	22.2	22.2
HIV-1 subtype B (%)	88.7	90.4
Trofile result (%)		
CCR5	71.4	100
CXCR4	2.7	0
D/M	25.9	0
Coprescribed antiretroviral drugs (%)		
Raltegravir	NA ^b	67.9
Darunavir	NA	53.6
Etravirine	NA	28.6
Enfuvirtide	NA	17.0
Median wGSS (IQR)	NA	1 (0.5–1)

^a MVC, maraviroc; IQR, interquartile range; NRTIs, nucleoside RT inhibitors; NNRTIs, nonnucleoside RT inhibitors; PIs, protease inhibitors; D/M, dual/mixed; wGSS, weighed genotypic sensitivity score.

^b NA, not applicable.

mination, because of nonavailability of plasma sample or failure of PCR amplification. Thus, 189 patients had both Trofile and gp120 sequence determination and were included in the study comparing genotypic and phenotypic determinations of coreceptor use. Within these 189 patients, 165 had the standard Trofile assay and 24 had the enhanced-sensitivity assay.

Within these 189 patients, 54 had a Trofile result of X4 or dual or mixed (D/M) and 135 were scored as R5 with Trofile and were treated with MVC. The correlation between VR and MVC and the baseline genotypic parameters was assessed in 112 patients with available follow-up. The patients' characteristics at screening and at baseline MVC appear in Table 1. The median CD4 cell count was 213 (interquartile range [IQR], 92 to 345) in patients with a Trofile result of X4 or D/M compared with 267 (IQR, 121 to 417) in patients with an R5 result (Wilcoxon test, *P* = 0.05). The median GSS was 1 (IQR, 0 to 1). The percentage of patients with GSS at 0 or 0.5 was 34%, while 44% of patients had a GSS of 1, and 22% of patients had

a GSS of 1.5. The nadir of the CD4 cell count was missing for 42 and 26 patients, respectively, among the 189 and 112 patients presented in Table 1.

Correlation between phenotypic and genotypic predictions of coreceptor use. The V3 loop sequence enabled the prediction of HIV-1 coreceptor use according to 14 genotypic algorithms or combinations of algorithms in 189 patients. The sensitivity and specificity of the prediction of X4 tropism in comparison with Trofile appear in Fig. 2 for the different algorithms. The highest sensitivity for individual algorithms was obtained with Geno2pheno at a 10% FPR using either the “clonal” or the “clinical” data set (both sensitivities of 59.3%). No increase in sensitivity was obtained when combining both Geno2pheno and PSSM algorithms. A high sensitivity (78.8%) was obtained with the global combination of all individual algorithms for the prediction of X4 viruses.

The specificity of the detection of X4 in comparison with Trofile was high (79.3% to 98.5%) for all individual algorithms or dual combinations of algorithms. A decrease of specificity (62.9%) was observed with the global combination of individual algorithms.

Virological and immunological responses to MVC. The VR to MVC was studied at M1, M3, and M6. The VR at M1 was obtained in 94/111 (85%) patients, the VR at M3 was obtained in 88/105 (84%) patients, and the VR at M6 was reached in 67/98 (68%) patients. At M6, the median decrease in plasma HIV-1

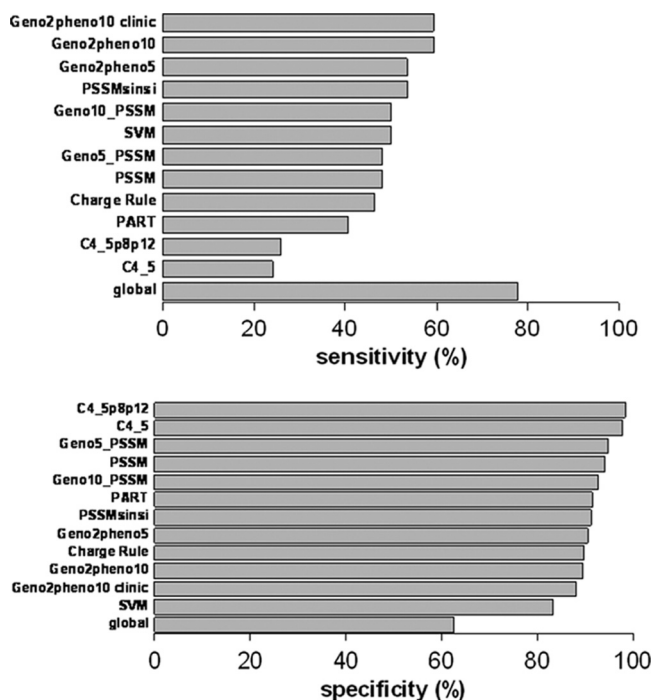


FIG. 2. Sensitivity and specificity of the detection of X4 or dual/mixed isolates according to 14 genotypic algorithms or combinations of algorithms compared to Trofile (*n* = 189 patients). Geno2pheno5 and Geno2pheno10, Geno2pheno algorithms with a false-positive rate set up at 10% or 5%, respectively; Geno2pheno10Clinic, Geno2pheno10 with “clinical” parameters (baseline HIV-1 RNA and nadir CD4⁺ cell count); Geno5PSSM or Geno10PSSM, combinations of Geno2pheno and PSSM algorithms; global, combination of all individual algorithms for detection of X4 or dual/mixed isolates.

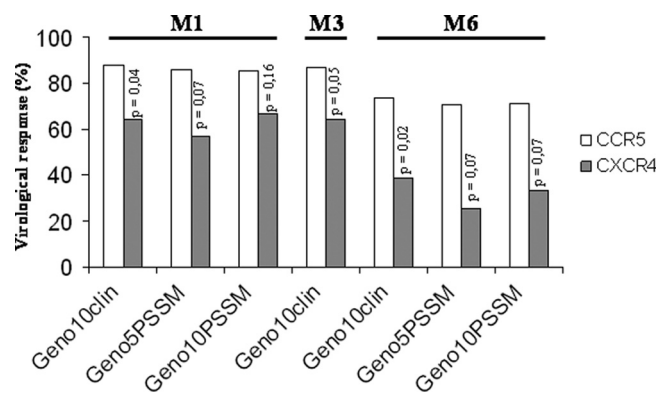


FIG. 3. Virological response to maraviroc-based regimens according to the baseline HIV-1 coreceptor use predicted by different genotypic algorithms ($n = 112$ patients). Geno10clin, Geno2pheno algorithm with false-positive rate set at 10% and with “clinical” parameters (baseline HIV-1 RNA and nadir CD4⁺ cell count); Geno5PSSM or Geno10PSSM, combinations of Geno2pheno (with false-positive rates set at 5% or 10%) and PSSM algorithms; M1, M2, and M3, date (in months) of follow-up.

RNA was $-2.34 \log_{10}$ copies/ml (IQR, -1.35 to -2.97) and the median increase in CD4⁺ cells was 81 cells/ μ l (IQR, 25 to 180).

Association between genotypic prediction of coreceptor use and virological response. The association between the genotypic prediction of coreceptor use and the VR to MVC plus optimized background therapy (OBT) was studied at M1, M3, and M6, by using the different genotypic algorithms or combinations of algorithms. The percentage of prediction of X4 tropism in the 112 studied patients with an R5 result with the phenotypic assay comprised between 2% and 21%, according to the different interpretation systems. Significant associations between genotypic coreceptor use and VR ($P < 0.05$) or trends

($P < 0.2$) are shown in Fig. 3. The percentage of VR was significantly higher in patients with baseline R5 according to the Geno2pheno10 algorithm with a clinical data set (Geno2pheno10Clin) at M1, M3, and M6. There was a trend toward association with VR for Geno2pheno5 plus PSSM_{R5X4} (Geno2pheno5+PSSM) and Geno2pheno10 plus PSSM_{R5X4} (Geno2pheno10+PSSM) at M1 and M6, respectively.

Baseline V3 loop mutations and virological response to MVC. The gp120 V3 loop amino acid substitutions were determined before treatment with MVC. Particular amino acid patterns, previously reported to be associated with phenotypic resistance to MVC and which had been shown to be polymorphic in MVC-naïve patients (15), were investigated. The prevalences of the different patterns were as follows: 11S plus 26V, 7.5%; 18G plus 22T, 1.0%; 19S plus 26V, 1.0%; 20F plus 25D plus 26V, 3.0%; 20F plus 21I, 0.5%; and 21T plus 28V, 0%. No significant association between the presence of these patterns at baseline and the subsequent VR to MVC-based regimens could be evidenced. We also searched for an association between all individual baseline V3 loop polymorphisms and VR; no significant association was found (data not shown).

Multivariate analysis of the factors associated with VR to MVC. In the univariate analysis (Table 2), the baseline coreceptor use prediction with Geno2pheno10Clin algorithm was at each time of follow-up was significantly associated with the VR to MVC. The nadir of CD4 cell count, the baseline of HIV RNA, wGSS (in which a wGSS of ≥ 1 was associated with a better VR), and the coreceptor use prediction with Geno2pheno5 plus PSSM were at some points of follow-up associated with the VR to MVC. As mentioned previously, information on the nadir of CD4 cell count is missing in 26 (23%) out of the 112 patients included in this analysis. We then built multivariate models with and without inclusion of the nadir variable in the stepwise selection procedure for each VR.

TABLE 2. Factors associated with the absence of virological response on maraviroc-containing regimens^a

Time of VR and variables	Univariate analysis		Multivariate analysis ^b			
			Without CD4 nadir		With CD4 nadir	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
M1						
Geno2pheno10Clin	3.94 (1.06–13.55)	0.041	3.94 (1.06–13.55)	0.041		
Geno2pheno5+PSSM	1.57 (0.97–23.87)	0.07				
Geno2pheno10+PSSM	3.14 (0.61–13.45)	0.16				
Nadir CD4	2.20 (1.04–5.54)	0.039				
M3						
Geno2pheno10Clin	3.66 (0.99–12.61)	0.052	3.66 (0.99–12.61)	0.052		
Baseline VL	1.45 (0.90–2.33)	0.12				
M6						
Geno2pheno10Clin	4.31 (1.31–15.57)	0.017				
Baseline VL	0.58 (0.34–0.91)	0.016			0.43 (0.21–0.86)	0.017
wGSS	3.31 (1.37–8.20)	0.008	3.31 (1.37–8.20)	0.008	3.15 (1.01–9.89)	0.049
Nadir CD4	2.98 (1.53–6.62)	0.0007			2.31 (1.06–5.00)	0.035
Geno2pheno5+PSSM	7.07 (0.86–146)	0.069				
Geno2pheno10+PSSM	4.81 (0.89–36.2)	0.069				

^a VR, virological response; OR, odds ratio; CI, confidence interval; M1, -3, and -6, number of months on MVC; Geno2pheno10Clin, baseline virus scored as X4 with the algorithm Geno2pheno with clinical parameters and with a false-positive rate (FPR) set at 10%; Geno2pheno5+PSSM and Geno2pheno10+PSSM, baseline virus scored as X4 either with the algorithm Geno2pheno with an FPR set at 5% or 10% or with the PSSM algorithm; Nadir CD4, nadir of CD4⁺ cell count/ μ l; Baseline VL, baseline viral load; wGSS, weighed genotypic sensitivity score.

^b Shown are results for the multivariate model without the CD4 cell count nadir and with the CD4 cell count nadir included in the stepwise selection procedure.

The multivariate logistic model without inclusion of the CD4 cell count nadir in the stepwise selection procedure retained only one variable for each distinct follow-up. Including the CD4 cell count nadir, no variables were retained in the association with VR at both month 1 and month 3. At month 6, a lower baseline HIV RNA, a wGSS of ≥ 1 , and a higher CD4 cell count nadir were independently associated with VR to MVC.

DISCUSSION

In the setting of the treatment of HIV-1 infection by CCR5 inhibitors, the possibility to use accurate and clinically validated tests for prediction of HIV-1 coreceptor use has become a priority. In this multicenter prospective study, we could evaluate the performance of genotypic tools in comparison with a phenotypic assay for the determination of coreceptor usage. Furthermore, we could study the relationship between the baseline genotype-predicted coreceptor use and the VR to MVC in antiretroviral-experienced patients.

We compared the prediction of coreceptor use by 13 different genotypic algorithms or combinations of algorithms with the Trofile phenotypic assay considered as the reference. We could show important differences between algorithms in the sensitivity of detection of X4 isolates. The most sensitive bioinformatic tools were PSSM and Geno2pheno, with sensitivities at about 60%. We found no evidence of increased sensitivity by combining these two algorithms for the detection of X4. In contrast, the specificity of the predicted X4 tropism was high for most algorithms. Similar performances of genotypic prediction compared to the standard Trofile assay have been reported after analysis of the MOTIVATE-1 Study database (P. R. Harrigan, R. McGovern, W. Dong, L. Swenson, A. Thielen, M. Jensen, T. Mo, D. Chapman, M. Lewis, I. James, J. Heera, S. Ellery, and H. Valdez, presented at the 5th IAS Conference on HIV Pathogenesis, Treatment and Prevention, CapeTown, South Africa, 2009). However, in other studies comparing phenotypic and genotypic predictions of coreceptor use, higher sensitivities of genotypic algorithms could be found (3, 13). Several factors can explain this somewhat low sensitivity of bioinformatic tools for the detection of X4 in our study. First, in our patients the percentage of X4 or D/M isolates at screening was quite low (28.8%), and higher sensitivities could have been found with a less rare X4-D/M event. Second, the sequences generated for genotypic analysis and for the Trofile assay corresponded to different PCR assays, which could have created some discrepancy; our study was a prospective, multicenter study, certainly more likely to evaluate the use of genotypic tools in routine diagnosis than monocentric studies on selected samples. Third, a minority of patients (24/189) had a Trofile assay of the enhanced-sensitivity version, which could have resulted in a better sensitivity than genotypic tests for detecting X4 isolates. However, no higher sensitivities of genotypic tests were found when considering only patients who had the standard Trofile assay. Fourth, considering the median viral load, the input copy number was relatively high in this study. However, it could be a limitation for the representation of viral diversity in patients with low viral load. Additional studies including the use of the triplicate versus single PCR and sequence are warranted in this setting. Finally, further

development of bioinformatic tools, including the analysis of viral determinants of coreceptor use outside of the V3 loop, could improve their sensitivity in the future.

Another important result of our study is the association between genotypic prediction of coreceptor use and VR to MVC-based regimens. We could show that the Geno2pheno algorithm using "clinical" parameters (baseline viral load and nadir of CD4⁺ cell count) in addition to the gp120 V3 loop sequence for predicting coreceptor use, could be predictive of the VR to MVC. The association between a better VR at months 1 and 3 on MVC and a predicted R5 coreceptor use was conserved in multivariate models which showed that a higher wGSS was also independently associated with a better VR at month 6. There was also a trend toward an association with VR for the combination of algorithms Geno2pheno and PSSM. The association between Geno2phenoClin and VR was not found in multivariate models, including the nadir CD4 count, probably because the nadir CD4 count was itself used for the prediction of coreceptor use with this algorithm. It was thus difficult to completely demonstrate the significance of prediction of VR by the coreceptor use prediction, because of the important predictivity of the nadir CD4 cell count and the overall good VR, due to MVC but also due to background therapy.

It is of importance that this clinical validation of the genotypic prediction of coreceptor use was assessed in a particular population of viruses which had first been screened as R5 by the Trofile assay. We thus showed that even in this biased population, genotypic tools can be associated with VR. In a larger, and more diverse viral population including patients screened as R5 but also found to be X4 or D/M by Trofile, Harrigan et al. (presented at the 5th IAS Conference on HIV Pathogenesis, Treatment and Prevention, CapeTown, South Africa, 2009) showed that genotypic tools (Geno2pheno and PSSM) could predict the VR to MVC-based regimens with an accuracy comparable to that of the Trofile assay. Both studies are thus in favor of the use of genotypic tools for coreceptor use determination in order to prescribe MVC in antiretroviral-experienced patients. Similar conclusions were also obtained from the Berlin Maraviroc Cohort (M. Obermeier, A. Carganico, T. Berg, B. Hintsche, S. Köppe, A. Moll, C. Mayr, B. Bienek, F. Scholte, and A. Baumgarten, presented at the 7th European HIV Drug Resistance Workshop, Stockholm, Sweden, 2009). One important point for determining the coreceptor usage with the Geno2pheno algorithm is the determination of the false-positive rate (FPR) for the detection of X4 isolates. A higher FPR enables the detection of more X4 viruses, but with less specificity. Our data suggest that an FPR of 10% leads to a good balance between sensitivity and specificity and shows the highest correlation with VR. Interestingly, a recent study of the MOTIVATE Trial suggested that a Geno2pheno score of 7.5% provided a good discrimination for the prediction of an optimal VR to MVC (P. R. Harrigan, R. McGovern, W. Dong, T. Mo, X. Zhong, D. Chapman, M. Lewis, A. Thielen, M. Jensen, H. Valdez, J. Heera, et al., presented at the 12th European AIDS Conference, Cologne, Germany, 2009).

Because of emerging evidence that the determinants of viral resistance to MVC (i.e., CCR5 use in the presence of the inhibitor) can be found predominantly within the V3 loop (2), we studied the prevalence of specific substitutions previously

associated with resistance to MVC in the baseline isolates before therapy including MVC. Although some patterns were found at a prevalence of 0.5% to 7.5%, no association between the presence of these substitutions at baseline and a poorer VR to MVC could be shown. Additional studies with higher statistical power should be set up in order to further document this question.

Other studies will be needed to further study the prediction of coreceptor use in HIV-1 non-B subtypes, since their prevalence was low in our patients. An initial report showed a poor performance of genotypic tools for non-B subtypes (6). However, two more recent studies are in favor of this approach for CRF02_AG and subtype C (12, 14).

In conclusion, our study provides both biological and clinical validation of genotypic tools for the prediction of HIV-1 coreceptor use in antiretroviral-experienced patients. Our results and other already cited concordant studies were the basis for the recommendation of genotypic tests in this setting by the French Health Agency (<http://www.has-sante.fr>) and by the European guidelines (www.europeanclinicalinfectiousociety.org/guidelines.asp). Genotypic tools for coreceptor usage will now be used to prescribe CCR5 inhibitors in antiretroviral-experienced patients. Further studies will be needed to clinically validate the genotypic prediction of coreceptor use before prescription of CCR5 inhibitors in antiretroviral-naïve patients or from HIV-1 DNA in patients with undetectable viremia.

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